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Laser-Mediated Reversal of Cardiac Expansion After Myocardial Infarction

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Background and Objectives: **A complication of transmural myocardial infarction is infarct expansion, which can lead to the development of heart failure. However, the necrotic muscle is replaced by collagen, a material known to shrink when heated. Thus, the hypothesis was that thermally-induced scar shrinkage could reverse infarct expansion.**
Study Design/Materials and Methods: **Four weeks after transmural infarcts were produced by coronary occlusion, rats were randomized to control or treatment with a neodymium:yttrium-aluminum-garnet laser. The epicardial scar surface was irradiated until shrinkage was observed. Thirty minutes later, hearts were excised and fixed at a distending pressure of 15 mm Hg, left ventricular cavity volume was measured, and histologic analysis was performed.**
Results: **Cavity volume was reduced by laser treatment (0.72 ± 0.07 ml vs. 0.54 ± 0.05 ml; *P* = 0.044). In addition, treatment resulted in thicker scars, a leftward shift of the heart's electrical axis, and straightening of collagen fibers.**
Conclusion: **Laser treatment resulted in thermally-mediated scar shrinkage, which reversed infarct expansion and reduced cavity volume. Lasers Surg. Med. 25:198–206, 1999.**

Key words: aneurysm; collagen; lasers; myocardial infarction; remodeling

INTRODUCTION

The presence of a large and transmural area of necrosis after myocardial infarction usually results in thinning of the necrotic segment, ventricular expansion, and cardiac shape changes. Such structural alterations lead to increases in ventricular cavity volume, which in turn are associated with the development of heart failure and increased mortality [1]. Drug treatments initiated soon after a heart attack have often been successful in limiting increases in cavity volume; however, these are therapies that cannot reverse changes that have already occurred and so, even with prolonged treatment, heart failure can develop. The muscle killed by infarction is eventually replaced by scar tissue, composed primarily of collagen. One physical property of collagen that has been exploited in other fields is its marked shrinkage when heated to temperatures above 70°C. Heating with lasers or radiofrequency devices has been used to treat joint laxity [2], while laser-induced collagen contraction plays a role in the removal of dermal wrinkles [3]. Furthermore, thermally-mediated collagen shrinkage has been used in the cornea to correct astigmatism [4]. Joint capsules, skin, and cornea all possess a high collagen content, a feature shared by myocardial scar tissue. Thus, the infarct scar represents an attractive target for thermally-induced shrinkage. In this initial examination of the concept, a single laser treatment to induce scar shrinkage immediately reversed the increases in ventricular cavity volume seen 4 weeks after infarction in rat hearts.

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MATERIALS AND METHODS

The protocol was approved by the hospital's Institutional Animal Care and Use Committee and conformed to the position of the American Heart Association on research animal use. The Heart Institute is accredited by the American Association for the Accreditation of Laboratory Animal Care.

Surgery

Female Sprague-Dawley rats were anesthetized by an intraperitoneal injection of ketamine (50 mg kg^{-1}) and xylazine (10 mg kg^{-1}), intubated, and ventilated with room air. A thoracotomy was performed through the fourth intercostal space and a stitch was taken from the atrioventricular groove to the pulmonary cone to encircle the left coronary artery, which supplies blood to the entire lateral wall of the left ventricle [5]. Arterial occlusion was achieved by tying a double knot in the suture. Four weeks later, the rats were reanesthetized and randomized to control or laser treatment ($n = 14$). In all animals, the chest was opened through the fifth intercostal space to expose the infarct. The electrocardiogram was recorded before and after treatment for each of the limb and augmented unipolar leads. These recordings were used to measure heart rate and detect ectopic beats, and were also examined for the presence of Q-waves, a marker of transmural infarction. The orientation of the heart's electrical axis (called the QRS axis) was determined from leads L_1 and aVF [6]. Arterial pressure was monitored by insertion of a fluid-filled catheter into the left femoral artery.

Laser Treatment

The beam from a pulsed neodymium:YAG (yttrium-aluminum-garnet; wavelength, 1.32 μm, pulse width, 250 μsec; Schwartz Electro-Optics, Inc., Orlando, FL) laser was focused into a flat-tipped 400-μm-diameter (320-μm core) fiber-optic. A pulse energy of 440 mJ was applied at 5 Hz (fluence at the fiber tip ≈ 550 J cm^{-2}). These parameters were chosen after experiments on tendon irradiated at a variety of energies and pulse rates to find a dose that caused shrinkage without charring. The fiber-optic was held approximately 5 mm from the epicardial surface (approximate spot size on epicardial surface 0.07 cm^2) and slowly swept back and forth across the scar, taking care to avoid irradiating viable muscle. The hearts were manipulated using a cotton-tipped swab so that all portions of the infarct, including the apex, could be irradiated. Irradiation exposures of approximately 10 seconds (i.e., 50 pulses/exposure) were applied until shrinkage was observed (this occurred after 1–3 exposures applied over a 10-minute period). Rats that were randomized to the control group underwent a 10-minute waiting period.

TABLE 1. Hemodynamics*

	Heart rate (bpm)		Mean arterial blood pressure (mm Hg)	
	Start	End	Start	End
Control	191 ± 10	185 ± 14	91 ± 11	76 ± 10
Laser	217 ± 13	173 ± 14	84 ± 7	66 ± 13

*bpm, beats per minute; mm Hg, millimeters of mercury.

Tissue Preparation

Thirty minutes after treatment or the waiting period, the hearts were arrested by injection of potassium chloride solution and excised, and the aortas were cannulated and attached to a column of formalin set to a height equivalent to a distending pressure of 15 mm Hg (to approximate the end-diastolic configuration). The cannulated hearts were also immersed in formalin. After fixation, heart mass was measured, and remeasured after the ventricular cavity had been filled with water to the level of the aortic valve, and the difference in mass was calculated to determine cavity volume. Histologic sections from all slices were cut in cross-section (thickness, 5 μm) and stained with picrosirius red [7]. Sections from a slice in the center of the infarct were projected, and the circumferential lengths occupied by scar and muscle were measured. Infarct size is typically expressed as the percentage of the left ventricular cross-sectional circumference occupied by scar; however, this method is not appropriate in an experiment where the scar is manipulated. Therefore, infarct size was represented by the circumferential length of noninfarcted muscle; the shorter the muscle length, the larger the infarct.

Histologic Analysis

The scar thickness was measured at one location in the center of the infarct in the picrosirius red-stained section used in the determination of infarct size. Measurements were made using a calibrated eyepiece reticle and a ×20 objective lens. The optical properties of collagen stained with picrosirius red and viewed with polarized light were exploited to assess the extent of laser-induced thermal changes and fiber organization.

Fig. 1. The untreated heart (right) was more spherical than the laser-treated heart. Although infarct size was similar and body mass identical, volume was 0.65 ml in the untreated and 0.47 ml in the laser-treated heart.

Collagen's anisotropic structure makes it birefringent and so it appears bright when viewed with polarized light. The degree of birefringence, and hence brightness, depends upon molecular anisotropy; the greater the anisotropy, the greater the brightness. Conversely, decreases in anisotropy result in decreased brightness [8]. The molecular structure of collagen is partially denatured, resulting in shrinkage at temperatures above 70°C. This denaturation reduces birefringence and brightness [9]. The depth to which fibers with reduced birefringence were seen (measured at the same location as scar thickness) was noted and expressed as a percentage of the total scar thickness. The two-dimensional orientation of longitudinally sectioned collagen fibers in each scar was measured, using a previously described polarized light technique [10]. Fiber orientation was measured at 50 locations at both the center and edge of each scar (in laser-treated hearts, a region where there was no reduction in birefringence).

Protocol 2: Echocardiography Study

To confirm that the volume reduction was not merely a function of differences in infarct size between groups, an additional four rats were examined. Myocardial infarction was induced as described above and the rats were reanesthetized 4–5 weeks later. Two-dimensional cross-sectional images of the left ventricle were obtained using a 7.5-MHz pediatric transducer connected to a echocardiographic computer console (Sonos 1000, Hewlett Packard, Corvallis, OR). Measurements of the end-diastolic and end-systolic left ventricular diameters were made with the chest closed. Laser treatment was then conducted as described above, the chest was closed, and the cavity diameters were remeasured.

Statistics

Data are expressed as mean ± standard error of the mean. Comparisons between groups were

TABLE 2. Cardiac Structural Changes

	LV cavity volume (ml)	Cavity area (cm^2)	Cavity diameter (mm)	Scar thickness (μm)	Collagen orientation Angular deviation (degrees)	
					Scar center	Scar edge
Untreated	0.72 ± 0.07	0.69 ± 0.05	9.2 ± 0.2	355 ± 40	10.1 ± 1.3	10.3 ± 0.4
Laser	0.54 ± 0.05	0.50 ± 0.04	8.3 ± 0.3	550 ± 50	4.8 ± 0.3	7.1 ± 0.6
P value	<0.05	<0.05	<0.01	<0.01	<0.001	<0.05

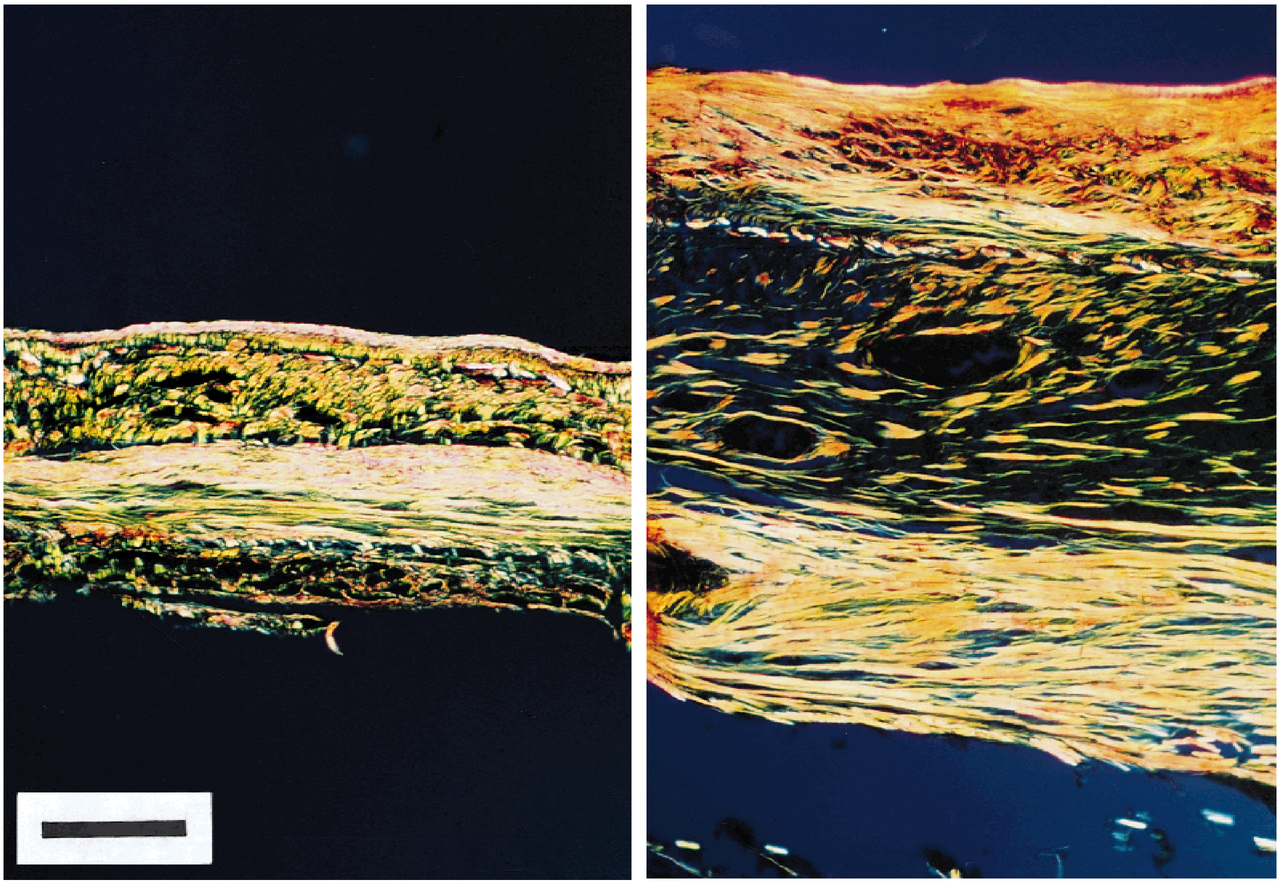

Fig. 2. Central scar regions from untreated (**left**) and laser-treated (**right**) hearts stained with picrosirius red and viewed with circularly polarized light (both printed at same magnification; epicardial surface at top). The untreated scar is thinner (200 μm) than the laser-treated scar (470 μm). Fibers in the subepicardial and midmyocardial regions of the laser-treated scar have reduced birefringence and appear dull, indicating that they were subjected to a temperature >70°C. Fibers in the subendocardium appear brighter and so were not subjected to the same temperature increase. The apparent elliptical gaps in the central region of the treated scar are nonbirefringent smooth muscle cells within intramyocardial arteries (bar = 100 μm).

made with two-tailed *t*-tests, except in protocol 2, where the hypothesis was that the diameters were reduced by treatment and so a one-tailed paired t-test was used. The two-dimensional orientation of collagen fibers was analyzed using circular statistics, i.e., a well-established branch of statistics developed to analyze variables such as direction angles that are not linear (for a detailed description, see Batschelet [11]). Means were considered to be different if the probability was <0.05.

RESULTS

Q-waves were present in lead L_1 of the electrocardiogram for all hearts prior to laser treatment, a finding consistent with transmural infarction. The presence of a transmural scar was confirmed by subsequent histologic analysis.

Hemodynamics

Treatment was associated with a drop in arterial pressure, sometimes to values of 20 mm Hg.

However, this reduction was transient and arterial pressure returned to pretreatment values within 2 minutes after irradiation. There were no differences in heart rate or mean arterial pressure between treated and untreated groups at any time during the experiment (Table 1).

Macroscopic Changes

There was no difference in the average fixed heart mass between groups (control, 1.04 ± 0.08 g; laser-treated, 1.06 ± 0.07 g; $P = 0.89$). Untreated infarcted hearts appeared spherical (Fig. 1). Laser treatment remodeled the heart and restored the prolate appearance of normal, noninfarcted hearts. This remodeling was accompanied by a 25% reduction in cavity volume and corresponding reductions in cross-sectional area and diameter vs. untreated, infarcted hearts (Table 2). Circumferential scar length in the middle of the infarct was reduced by laser treatment (8.9 ± 0.6 mm vs. 10.9 ± 0.9 mm, $P = 0.045$), but muscle length was the same in both groups (12.1 ± 1.0 mm vs. 11.6 ± 1.1 mm; $P = 0.72$), consistent with equal infarct sizes.

Microscopic Changes

Scar thickness was increased in laser-treated hearts (Table 2, Fig. 2). Reductions in collagen birefringence extended from the epicardial surface to a depth of 69 ± 5% of the entire scar thickness. Qualitative inspection (Fig. 3), as well as quantitative analysis, revealed that collagen fibers in laser-treated scars were straighter than those in untreated scars. Figure 4 shows representative two-dimensional fiber orientation distributions for both groups. Distributions obtained from the center and edge of untreated scars were similar. In contrast, distributions obtained from the central, irradiated regions of laser-treated scars were tighter (the fibers more aligned) than in the nonirradiated regions at the scars' edge. When the average angular deviation, a measure of the spread of the distribution, was calculated for all scars (Table 2), collagen fibers were always more coherently aligned (the angular deviation was smaller) in laser-treated scars than in the corresponding region of untreated scars.

Electrocardiographic Changes

Laser irradiation caused arrhythmias, in the form of ventricular premature beats and some short runs of ventricular tachycardia. However, normal sinus rhythm was restored as soon as treatment ended. No ectopic beats were observed

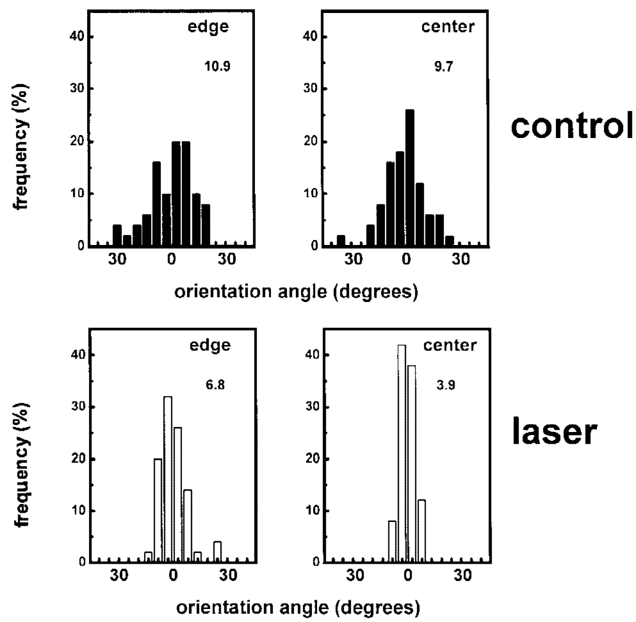

Fig. 3. Collagen fiber orientation obtained from the edge and center of scars in an untreated (**above**) and laser-treated heart (**below**). The x-axis shows the orientation angle; each division represents 5°, and zero indicates the mean of each distribution. The y-axis shows orientation frequency. The value in the upper right corner of each image is the angular deviation of the distribution. The distributions obtained from both regions of the control scar are similar and are spread over a range of 60°. In contrast, the distribution from the center of the laser-treated scar is contained within a range of 20°.

in the control hearts. Additional evidence for structural remodeling induced by laser treatment was provided by electrocardiogram analysis. Myocardial infarction causes a shift in the QRS axis from the 0–90° quadrant to the 90–180° quadrant [6]. Laser treatment shifted the QRS axis back from 117 ± 13° to 67 ± 13° ($P = 0.03$). The orientation of the QRS axis in untreated hearts (107 ± 27°) was not different from the pretreatment value in the laser group. Q-waves were observed in lead L_1 for all hearts; however, they were no longer present after laser treatment in three hearts. In these cases a "new" Q-wave was found in another lead (aVR and/or lead L_2) that contained no Q-wave before treatment.

Echocardiography

The mean end-diastolic diameter of the left ventricular cavity decreased from 9.6 ± 1.1 cm to 8.4 ± 1.0 cm after laser treatment ($P = 0.014$). The end-systolic diameter also decreased after laser treatment from 6.4 ± 1.0 cm to 5.2 ± 0.5 cm ($P = 0.039$).

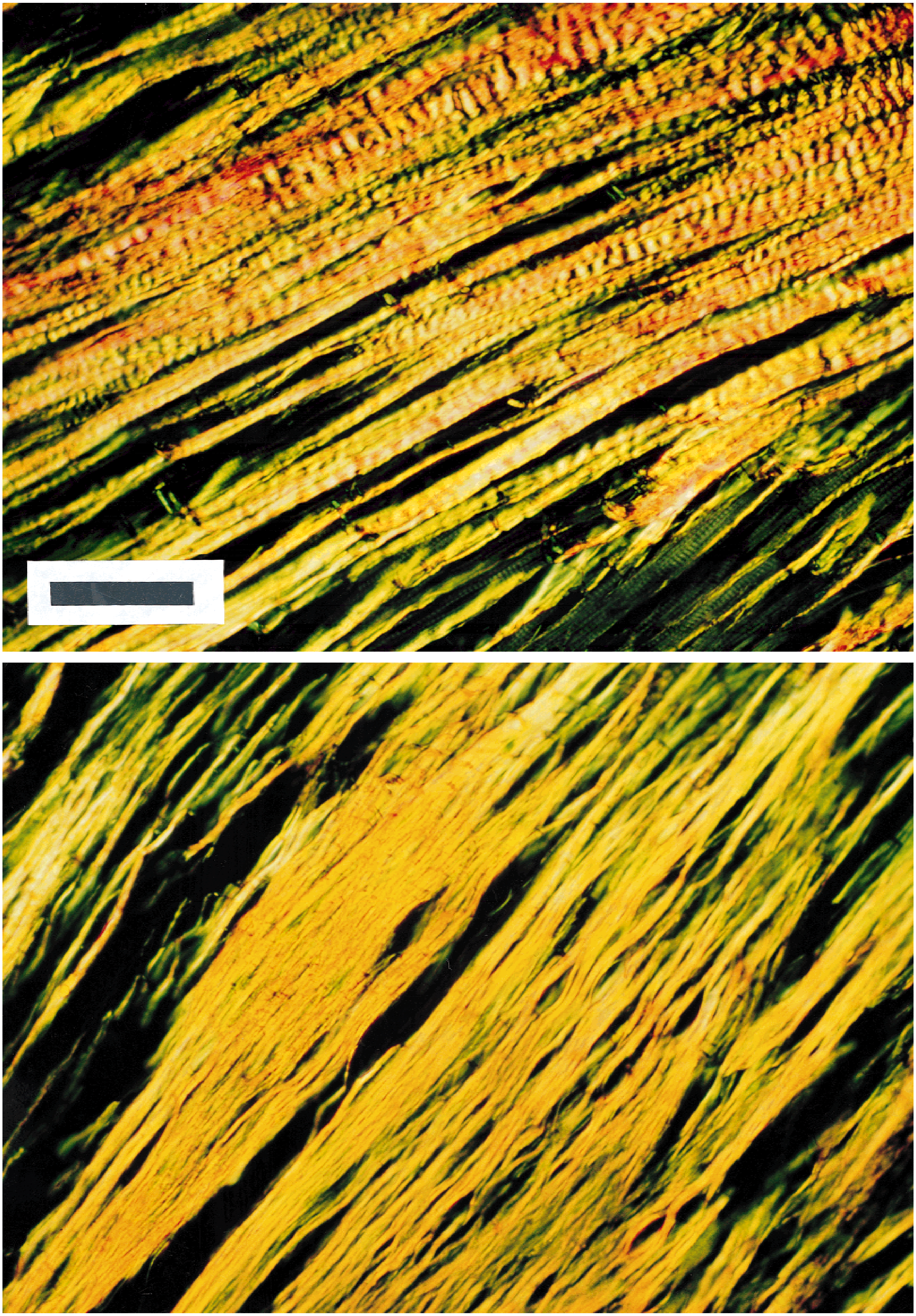

Fig. 4. Collagen at the edge of untreated (**above**) and laser-treated (**below**) scars stained with picrosirius red and viewed with linearly polarized light. Untreated fibers have a banded appearance indicating waviness, while straight laser-treated fibers lack this banding (bar = 50 μm).

DISCUSSION

Laser irradiation of scar tissue successfully reversed ventricular expansion associated with transmural infarction. Although this reversal was immediate, the long-term effects have yet to be examined. Treatment not only significantly reduced ventricular volume and increased scar thickness, but also favorably changed ventricular shape. The spherical ventricular structure and blunting of the apex, which are associated with compromised ventricular function, were replaced by a more prolate ventricle with a sharper apex. Microscopic analysis revealed that these architectural changes were the result of thermally-mediated shrinkage of collagen fibers within the scar. Shifts in the QRS axis after treatment were also consistent with scar shrinkage. If the reversal of unfavorable infarction-mediated remodeling by laser treatment is maintained, this method has the potential to reduce the incidence of heart failure.

Structural Basis for Thermal Remodeling of the Heart

Evidence for scar shrinkage was provided by the appearance of collagen within the scar. In contrast to their usual wavy appearance, collagen fibers in laser-treated scars were straight. Nonirradiated fibers at the edges of laser-treated scars were also straighter than normal (Fig. 3). This finding suggests that such fibers were connected to those shortened and straightened directly by the temperature increase. These changes at the fiber level are consistent with the gross reduction in scar length.

Further evidence for scar and ventricular remodeling came from the observed shift in the QRS axis. The shift produced by laser treatment put the axis back in the range reported for normal rat hearts [12]. In addition, laser treatment sometimes removed the Q-waves that were observed prior to treatment. However, these findings do not mean that muscle was resurrected, but rather indicate that scar shrinkage altered cardiac geometry and shifted its position relative to the limb leads in a similar manner to axis shifts that can be caused by pregnancy or by the presence of large abdominal tumors [6]. The observed electrocardiographic changes may provide quantitative indicators of successful shrinkage superior to the subjective visual inspection method used in this experiment.

Cavity dimensions were measured only at the end of protocol 1, and therefore it is crucial to eliminate the possibility that cavity volume was less after treatment because it had been smaller beforehand. The two main factors that influence cavity volume are body mass and infarct size. Body mass is proportional to heart mass and therefore will also be proportional to cavity volume. However, the difference in initial body mass between groups was only 4%. Large infarcts are associated with increased cavity volume in rats [13]. The usual way of assessing infarct size is to calculate the proportion of the circumference of a ventricular cross-section occupied by the scar; however, if scar shrinkage has occurred, this method cannot be used. In contrast, the circumferential muscle length in a ventricular cross section should be unaffected by treatment and so serves as a proxy for infarct size. On this basis, infarct size differed by only 4% between groups. The lack of a difference in heart mass was also consistent with similar infarct sizes. Thus, the 25% reduction in cavity volume was the direct result of laser treatment. Further evidence to support this conclusion was obtained from four additional infarcted hearts examined using echocardiography before and after laser treatment. Both the end-diastolic and end-systolic diameters were smaller after treatment. Although the resolution of the echo images was less than that of the ex vivo measurements presented in Table 2, the data are consistent.

When Could Treatment Be Initiated?

Tissues successfully treated with thermally-induced shrinkage (joint capsules, skin, cornea) are characterized by high collagen content. Presumably for thermal shrinkage to be successful, the tissue must be primarily composed of a shrinkable material (i.e., collagen). Preliminary in vitro experiments revealed that normal uninfarcted hearts or hearts treated 2 days after infarction did not shrink during laser irradiation. Although normal hearts contain collagen, it is only a small amount (2–4% vs. ~60% in 4-week-old scars), and appeared insufficient to enable tissue contraction. Similarly, at 2 days after infarction, there will be little or no new collagen production and the content will be approximately the same as in uninfarcted hearts. Thus, in the infarcted heart, a waiting period is required before the collagen content has increased enough for

shrinkage to occur. This requirement indicates that treatment could be initiated at any time several weeks after infarction.

Other Surgical Methods of Cavity Volume Reduction

In addition to pharmacologic approaches, significant ventricular expansion may be treated surgically by a combination of scar excision and plication to reduce cavity volume [14]. However, such methods can distort the heart, and so attempts have been made to reshape the ventricle so that it resembles an infarcted ventricle in which no expansion has occurred by tailoring a prosthetic patch to match the size and geometry of the original infarct [15]. Partial left ventriculectomy is another procedure designed to reduce cavity volume; however, what is resected is a wedge of cardiac muscle rather than scar tissue. In fact, an inverse correlation between left ventricular performance after surgery and fibrosis indicates that, in contrast to thermal shrinkage, the presence of extensive collagen is a contraindication for this procedure [16]. The laser-mediated shrinkage method is similar in principle to these approaches, but avoids either the introduction of foreign materials or the need to cut open the ventricle.

Potential Limitations

Although immediate shrinkage was achieved, the long-term consequences are unknown. For example, the scar's mechanical properties may have been altered. It is known that tendons exposed to laser irradiation sufficient to cause shrinkage have reduced tensile strength [17]. However, even the reduced strength was sufficient to withstand physiologic stresses, and so it is unlikely that the relatively low stresses in the ventricle would be a problem. Furthermore, the extent of the laser's thermal effect in the heart, as indicated by reduced collagen birefringence, never extended through the entire scar thickness, and so a subendocardial band of unaffected collagen was always present, with presumably normal mechanical properties.

The laser treatment was restricted to scar tissue, and so no muscle outside the scar was injured (muscle injury would also be marked by a reduction in birefringence); however, scar shrinkage is the result of partial denaturation of the collagen fibers. In addition, there is likely to be dehydration within the scar. It would be important to determine how such changes influence healing of this thermal injury, particularly the length of time required.

These issues clearly need to be addressed before scar shrinkage is used to treat patients. Nevertheless, the reversal of infarct expansion by laser treatment has been demonstrated. This finding appears worthy of further preclinical investigation to determine if the acute structural changes can be maintained and translated into improvements in function and survival.

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